# POPULATION STRUCTURE IN A SNAIL SPECIES FROM ISOLATED MALAYSIAN LIMESTONE HILLS, INFERRED FROM RIBOSOMAL DNA SEQUENCES 

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#### Abstract

We sequenced the first internal transcribed spacer (ITS-1) of the ribosomal DNA in nine populations of the vertiginid Gyliotrachela hungerfordiana, which lives on isolated (and threatened) limestone hills in the Malaysian peninsula. Current data suggest that the species is an obligate calcicole. The application of a tentative molecular clock suggests a Quaternary divergence for the $G$. hungerfordiana populations. A strong positive correlation between genetic and geographic distance was observed, which, combined with geological data, suggests that the hill populations may be interconnected by as yet unsampled populations. Key words: internal transcribed spacer, ITS-1, Gastropoda, Pulmonata, Vertiginidae, Gyliotrachela, gene flow, Southeast Asia.


## INTRODUCTION

Land snails have proverbially poor abilities for dispersal (e.g., Cowie, 1984; Schilthuizen \& Lombaerts, 1994), which causes them to show evolutionary patterns at much smaller spatial scales than many other organisms of similar size. As a result, strong geographic structuring of populations is common in snails (e.g., in Liguus; Hillis et al., 1987). Another consequence is endemism, which is seen, for example, in the Mediterranean clausiliid genus Albinaria, of which almost 30 species are endemic to the island of Crete, with distribution areas of sometimes only one kilometer across (Gittenberger, 1991; Welter-Schultes, 1998).

An impressive situation of high endemism and geographic structuring of land snails in a strongly fragmented habitat exists in peninsular Malaysia. Here, limestone is exposed in the form of "tower karst" and other karstifications, limited to about three hundred hills, scattered over the peninsula. These hills are often very small, the largest with a diameter of a few kilometers, but most measuring only a few hundred meters across. In spite of their small size, the hills are a prominent feature of the landscape, because they usually stand isolated, are riddled with caves and are bounded by precipitous cliffs.

For more than a century, malacologists have been interested in the rich malacofauna that the hills support (de Morgan, 1885). High numbers of species are found, and the morphologies of some Diplommatinidae foreshadow the bizarre and extravagant forms found in this group in Borneo (Vermeulen, 1993, 1994; Gittenberger, 1995). But especially fascinating is the staggering degree of endemism in these calcicolous snails. Tweedie (1961) gave an overview of six taxa containing many obligate calcicoles (Diplommatina, Opisthostoma, Vertiginidae, Discartemon, Oophana, and Sinoennea). He listed the presence of 106 species on 28 hills or hillclusters, of which 70 are endemic to only one locality. Some calcicolous species, however, are widespread and occur on almost all hills without a trace of morphological differentiation (e.g. Gyliotrachela hungerfordiana and some Alycaeus species).

Geologically, the hills form the exposed parts of a number of larger paleozoic limestone deposits, which are elsewhere overlain by non-calciferous alluvial deposits (Gale, 1986; Crowther, 1986). Some hills may thus have been connected in the past, while others have always been separate. Consequently, the hills form virtual "islands" for obligately calcicolous land snails, which they may reach by incidental dispersal. Alternatively, the pop-

[^0]ulations on the hills may be relicts from a time when the hills were part of large continuous plateaus, which were subsequently fragmented.

In this paper, we examine a relatively widespread representative of the peninsular Malaysian hill malacofauna, using molecular and geological data, to answer the following questions: (1) what pattern of phylogeographic relationships exists among the populations of this widespread species, and (2) how has the population structure been shaped, that is, what are the relative influences of dispersal and habitat fragmentation over geological time?

By analyzing the variance in a noncoding nuclear DNA marker, we attempt to differentiate between various alternative population structures. In the case of ancient vicariance, we expect to find genetic distances that reflect the age of fragmentation of the limestone hills, while dispersal would result in genetic distances more or less related to geographic distance. Under the latter hypothesis (dispersal), indications of the type and frequency of dispersal may be gleaned from the degree of correlation between genetic and geographic distance; if dispersal is randomly oriented (i.e., corresponding to an island model of population structure; Wright, 1931), stochasticity would result in a poor fit, while dispersal occurring mainly among neighboring hills (i.e., corresponding to a stepping-stone model; Kimura, 1953) would be revealed by a strong correlation (Kimura \& Weiss, 1964).

Sadly, there are other motives for working on this fauna. The hills of peninsular Malaysia are disappearing and becoming depauperate at an alarming rate. Forest clearing has destroyed the vegetation on some hills; and in the densely populated areas near Ipoh and Kuantan, many hills are being removed by quarrying. The true rate of species loss can only be guessed at, but the extinction of at least one endemic snail species, Opisthostoma sciaphilum, from Bukit Panching, has been documented (Schilthuizen et al., unpubl.).

## MATERIAL AND METHODS

## Selection of Taxa

We selected the widespread and morphologically uniform vertiginid Gyliotrachela hungerfordiana for study (Fig. 1). The related


FIG. 1. Gyliotrachela hungerfordiana (von Möllendorff). Scale bar $=1 \mathrm{~mm}$.


FIG. 2. Gyliotrachela frequens van Benthem Jutting. Scale bar $=1 \mathrm{~mm}$.
species G. frequens (Fig. 2) was selected to serve as an outgroup in the phylogenetic analysis.

Collecting
In July 1997, the first author visited 22 limestone hills in the West-Malaysian states of Pahang, Kelantan, Perak and Perlis. Living snails were discovered by eye using two strategies: (a) close inspection of limestone rock faces,
either damp or dry, bare or covered in algae, mosses and lichens; and (b) sifting through damp and decaying leaf litter on limestone rocks or at the base of the limestone cliffs. All snails were put in $100 \%$ ethanol on the spot and kept at ambient temperatures until arrival in the laboratory for further processing. Identification of the material was carried out by the second author while the material remained in alcohol. Gyliotrachela hungerfordiana was collected from nine of the 22 localities (Fig. 3): loc. 5, State of Pahang: Gua Bama (ca. 10 km W of Kuala Lipis); loc. 8, State of Kelantan: Gua Musang, southern of the two hills that the road to Kuala Kerai passes between; loc. 9, State of Kelantan: rocks 59 km in the direction of Gua Musang, measured along the road from Kuala Krai; loc. 16, State of Perak: Bukit Tambun (ca. 6 km E of lpoh); loc. 22, State of Perak: hill directly east of Sungai Siput Utara hospital; loc. 23, State of Perlis: hill ca. 1 km S of Kangar; loc. 24, State of Perlis: 9 km along the road from Kangar to Kaki Bukit; loc. 25, State of Perlis: Gua Kelam at Kaki Bukit; loc. 26, State of Perlis, Timah Tasoh (ca. 16 km NE of Kangar). All samples were taken between 27.vi. 1997 and 17.vii.1997. Gyliotrachela frequens was taken only from locality 8 . Voucher specimens have been deposited in the collection of the National Museum of Natural History "Naturalis", Leiden.

## Molecular Techniques

DNA was isolated from pools of between one and five complete snails with their shells, using either a phenol/chloroform extraction as described previously (Schilthuizen et al., 1998a) or a sucrose-based protocol (van Moorsel \& van Nes, unpublished), which can be briefly summarized as follows. Snails were ground in $200 \mu$ l of sucrose-buffer ( 0.1 M Tris; $0.02 \mathrm{M} \mathrm{NaCl} ; 0.2 \mathrm{M}$ sucrose; 0.05 M EDTA) and centrifuged. The pellet was incubated at $65^{\circ} \mathrm{C}$ for 60 min in $200 \mu \mathrm{I}$ SDS-buffer ( 0.02 M Tris; 0.01 M EDTA, $1.25 \%$ SDS), $15 \mu \mathrm{l}$ of cold KAc was added, and the mixture was incubated on ice for 60 min and centrifuged. The DNA was precipitated from the supernatant by the addition of two volumes of $100 \%$ ethanol and incubation at $-20^{\circ} \mathrm{C}$ for 30 min . The DNA was dried and treated with 200 ng of RNase. Full details can be obtained from M.S. on request. Homogenization was always done with a sterile, disposable plastic pestle. The DNA was dissolved in $50 \mu$ l of Tris-EDTA buffer (phenol protocol) or $30 \mu \mathrm{l}$ of $\mathrm{ddH}_{2} \mathrm{O}$ (sucrose
protocol) and stored at $-20^{\circ} \mathrm{C}$. The first internal transcribed spacer of the nuclear ribosomal DNA was amplified with the SuperTaq enzyme (HT Biotechnology, Cambridge, England) as described previously (Schilthuizen et al., 1995) and isolated using the "freezesqueeze" technique (Tautz \& Renz, 1983). Because PCR-amplification was at times too weak for direct sequencing, we resorted to cloning (PCR-based error is usually not a concern with this methodology; Schilthuizen et al., 1998b). After isolation, the fragments were ligated into Promega or Invitrogen T-tailed vectors, following the manufacturer's instructions. Colonies were screened for the presence of the correct insert by PCR. Plasmid DNA was isolated from the bacteria using QIAPrep spin columns (QIAGEN). One or two clones per sample were sequenced in both directions on an ABI automated sequencer.

## Alignment

Before alignment, all chromatograms were checked and reading errors were corrected blindly where necessary (this never amounted to more than three corrections in a single sequence). Vector and primer sequences were removed. Sequences in the ingroup were sufficiently similar to allow manual alignment. Wherever alignment with the outgroup was ambiguous, missing data were introduced into the outgroup sequence.

## Phylogenetic Analysis

Phylogenetic analyses of the data set were performed in PAUP3. 1 (Swofford, 1993). Gaps were treated as missing data. Searches for the most parsimonious trees were carried out with the branch-and-bound option. Bootstrap replicates were carried out 100 times, using heuristic searches. In addition, Bremer (1988) support was determined. Kimura's 2-parameter genetic distances (Kimura, 1980) were calculated with the DNADIST program of the PHYLIP package (Felsenstein, 1995).

## RESULTS

PCR-products ranged in length from 755 to 772 bp , including primers ( 52 bp ), and the flanking regions of $18 \mathrm{~S}(146 \mathrm{bp})$ and 5.8 S ( 87 $\mathrm{bp})$. These lengths correspond well with other ITS-1 lengths reported in mollusks (Anderson


FIG. 3. A map of the northern part of Peninsular Malaysia, with the limestone hills drawn in black (modified after Gobbett, 1965). The numbers refer to localities where Gyliotrachela hungerfordiana and G. frequens were collected (see text for further details).
\& Adlard, 1994; Schilthuizen et al., 1995; Armbruster et al., unpubl.).

We obtained sixteen sequences from $G$. hungerfordiana and one sequence for the outgroup, G. frequens (Appendix, Table 1). They have been deposited in GenBank under accession numbers AF118000-AF118016. Only small genetic distances were found among the $G$. hunderfordiana sequences, the largest being 0.048 between sequence a from locality 5 and sequence $b$ from locality 23. A comparison between pairwise genetic distances and pairwise geographic distances between sequences revealed a strongly significant ( $\mathrm{p}<0.005$ ) positive correlation (Fig. 4, Appendix, Table 2). The phylogenetic analysis produced 18 most parsimonious trees (length $=89$ steps, $\mathrm{RI}=0.95$ ), which showed two
alternative topologies for three monophyletic groups of sequences, and otherwise only minor differences in topology within each of these three monophyletic groups (Figs. 5, 6). The fact that duplicate sequences from a single locality always formed monophyletic groups might justify the small sample sizes. Geographic structuring is apparent in the trees also, as these show monophyly for the sequences derived from populations in Perlis, Pahang + Kelantan, and Perak.

## DISCUSSION

Unfortunately, it is difficult to estimate reliably from the molecular data the time since divergence. Unlike the situation for mitochon-


FIG. 4. The relationship between geographic distance and Kimura's 2-parameter distance for the sequences of Gyliotrachela hungerfordiana.
drial DNA, corroborated molecular clocks for the ITS regions are hardly available yet, and where they are, they differ by orders of magnitude among taxonomic groups. In the angiosperm families Cucurbitaceae and Winteraceae, substitution rates of $3.62 \times 10^{-3}$ and $3.4 \times 10^{-4}$ per site per million years (MY) were calculated, respectively (Jobst et al., 1998; Suh et al., 1993), while in Chlorophyta, a rate of $0.8-2.0 \times 10^{-2}$ was estimated (Bakker et al., 1995). In animals, rates of substitution in ITS appear to be somewhat higher. Schlötterer et al. (1994) give a figure of $1.2 \times$ $10^{-2}$ for Drosophila, and preliminary data for clausiliid land snails from Greek islands indicate a similar rate (van Moorsel, unpublished data).

Here, we will adopt a substitution rate of $1 \times$ $10^{-2}$ per site per MY as a very rough molecular clock. Applying this rate to the average genetic distance between sequences on either side of the node basal to all $G$. hungerfordiana sequences in the trees, we obtained an estimated divergence time of 1.8 MYA for the populations of $G$. hungerfordiana. It should be stressed that, given the lack of agreement in the few calibrated molecular clocks available, not too much confidence should be placed on this date. However, it may be safe to assume a Late Tertiary or Quaternary origin for $G$. hungerfordiana.

Given the low degree of genetic divergence among the G. hungerfordiana populations, it seems unlikely that vicariance has played an important role; hills which have been studied geologically are thought to be older than Late Tertiary/Quaternary (Gale, 1986). However, in view of the uncertainty about the calibration of the ITS-1 molecular clock, this reasoning may be little meaningful. More importantly, geological data indicate that most of the hills from which the species was sampled have never been part of one continuous plateau (Paton, 1961). It is for this reason not likely that vicariance events have been important in its distribution pattern. Rather, the limestone hills on which it lives now must have been colonized after dispersal.

Several mechanisms for passive dispersal in small snails have been suggested, including wind and water mediated dispersal. In reference to Gyliotrachela and similar snails, Tweedie (1961) has suggested that flooding may be important in producing dispersal among hills that are situated close together. However, the drainage patterns in the peninsula preclude any long-range dispersal by this mechanism. Stagnant water may also provide means of dispersal, and geological data (Gale, 1986; Crowther, 1986) indicate that lacustrine conditions have prevailed around several limestone hills in the past. But here, too, dispersal would be across very small distances. Another possibility is wind-dispersal. Kirchner et al. (1997) demonstrate how Truncatellina, a vertiginid very similar in size to $G$. hungerfordiana, could be blown over distances of several kilometers during storms.

Some additional characteristics of dispersal may be gleaned from Figure 4 , which suggests a linear relationship between geographic and genetic distance. If dispersal from one hill to another were infrequent and undirected (i.e., a population structure corresponding to Wright's [1931] island model, where all possible pairs of subpopulations are equally likely to exchange migrants), such a clear relation would not be expected. The fact that genetic distance is reliably predicted ( $r^{2}=0.77$ ) by geographic distance, suggests that a structured network of dispersal connects the hills. This corresponds to a stepping-stone model (Kimura, 1953). Under such a model, genetic similarities drop steeply with increasing numbers of intervening populations (Kimura \& Weiss, 1964). The fact that we observe a strong relationship with geographic distance, suggests that the hill popu-


FIG. 5. A representative most parsimonious tree of the Gyliotrachela sequences. Bootstrap percentages and decay indices have been indicated on the branches.


FIG. 6. Strict consensus over all 18 most parsimonious trees.
lations cannot represent directly adjacent populations in a two-dimensional stepping-stone lattice. Rather, to obtain this result, it is necessary to postulate unsampled populations in between. Unfortunately, the population genetics of ribosomal DNA are as yet far from clear (Hillis et al., 1991; Rich et al., 1997), which makes a quantitative analysis of dispersal parameters and spatial details of the population structure impossible. Therefore, it is not possible to tell whether the hills that separate our sample sites (e.g., the six or more hills between sites 8 and 9 ) will suffice as additional stepping stones. This might be tested, for instance, by exhaustively sampling the hills in a given subregion.

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APPENDIX

TABLE 1. Aligned sequences for Gyliotrachela hungerfordiana and G. frequens. The $5^{\prime}$ end of the 18 S region is at position 146 , the $3^{\prime}$ end of the 5.8 S region is at position 694.


AGCGGTTCAG
AGCGGTTCAG AGCGGTTCAG AGCGGTTCAG AGCGGTTCAG AGCGGTTCAG AGCGGTTCAG AGCGGTTCAG AGCGGTTCAG AGCGGTTCAG AGCGGTTCAG AGCGGCTCAG AGCGGTTCAG AGCGGTTCAG AGCGGTTCAG AGCGGTTCAG AGCGGTTCAG
20 TGAGGGCCTC GGATTGGTCT CGGTCTGGTG CGCAAGTGCC TGAGGGCCTC GGATTGGTCT CGGTCTGGTG CGCAAGTGCC TGAGGGCCTC GGATTGGTCT CGGTCTGGTG CGCAAGTGCC TGAGGGCCTC GGATTGGTCT CGGTCTGGTG CGCAAGTGCC TGAGGGCCTC GGATTGGTCT CGGTCTGGTG CGCAAGTGCC TGAGGGCCTC GGATTGGTCT CGGTCTGGTG CGCAAGTGCC TGAGGGCCTC GGATTGGTCT CGGTCTGGTG CGCAAGTGCC TGAGGGCCTC GGATTGGTCT CGGTCTGGTG CGCAAGTGCC TGAGGGCCTC GGATTGGTCT CGGTCTGGTG CGCAAGTGCC TGAGGGCCTC GGATTGGTCT CGGTCTGGTG CGCAAGTGCC TGAGGGCCTC GGATTGGTCT CGGTCTGGTG CGCAAGTGCC TGAGGGCCTC GGATTGGTCT CGGTCTGGTG CGCAAGTGCC TGAGGGCCTC GGATTGGTCT CGGTCTGGTG CGCAAGTGCC TGAGGGCCTC GGATTGGTCT CGGTCTGGTG CGCAAGTGCC TGAGGGCCTC GGATTGGTCT CGGTCTGGTG CGCAAGTGCC TGAGGGCCTC GGATTGGTCT CGGTCTGGTG CGCAAGTGCC TGAGGGCCTC GGATTGGTCT CGGTCTGGTG CGCAAGTGCC
$\xrightarrow{60}$
0 - 70 - 90 100

GGCACCGCTG GCCGAGAAGA AGCTCGAACT CGATCGCTTG GAGAAAGTAA GGCACCGCTG GCCGAGAAGA AGCTCGAACT CGATCGCTTG GAGAAAGTAA GGCACCGCTG GCCGAGAAGA AGCTCGAACT CGATCGCTTG GAGAAAGTAA GGCACCGCTG GCCGAGAAGA AGCTCGAACT CGATCGCTTG GAGAAAGTAA GGCACCGCTG GCCGAGAAGA AGCTCGAACT CGATCGCTTG GAGAAAGTAA GGCGCCGCTG GCCGAGAAGA AGCTCGAACT CGATCGCTTG GAGAAAGTAA GGCACCGCTG GCCGAGAAGA AGCTCGAACT CGATCGCTTG GAGAAAGTAA GGCACCGCTG GCCGAGAAGA AGCTCGAACT CGATCGCTTG GAGAAAGTAA GGCATCGCTG GCCGAGAAGA AGCTCGAACT CGATCGCTTG GAGAAAGTAA GGCACCGCTG GCCGAGAAGA AGCTCGAACT CGATCGCTTG GAGAAAGTAA GGCACCGCTG GCCGAGAAGA AGCTCGAACT CGATCGCTTG GAGAAAGTAA GGCACCGCTG GCCGAGAAGA AGCTCGAACT CGATCGCTTG GAGAAAGTAA GGCACCGCTG GCCGAGAAGA AGCTCGAACT CGATCGCTTG GAGAAAGTAA GGCACCGCTG GCCGAGAAGA AGCTCGAACT CGATCGCTTG GAGAAAGTAA GGCACCGCTG GCCGAGAAGA AGCTCGAACT CGATCGCTTG GAGAAAGTAA GGCACCGCTG GCCGAGAAGA AGCTCGAACT CGATCGCTTG GAGAAAGTAA GGCACCGCTG GCCGAGAAGA AGCCCGAACT CGATCGCTTG GAGAAAGTAA
hungerfordiana.loc5\#a hungerfordiana. loc5\#b hungerfordiana.loc8\#a hungerfordiana. loc8\#b hungerfordiana. loc9\#a hungerfordiana.loc16\#a hungerfordiana.loc16\#b hungerfordiana. loc22\#a hungerfordiana. loc22\#b hungerfordiana.loc23\#a hungerfordiana.loc23\#b hungerfordiana.loc24\#a hungerfordiana.loc 24 \#b hungerfordiana.loc25\#a hungerfordiana.loc25\#b hungerfordiana.loc26\#a frequens

TABLE 1. Continued.

|  | $\stackrel{160}{1}$ |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: |
| hungerfordiana.loc5\#a | tat | TCA | GGCTGCAGCG | CGCAG | GCttatca |
| hungerfordiana.loc5\#b | TATAAT---- | -----CATCA | GgCAgCAGCG | gGgCecgcag | cgecttatga |
| hungerfordiana.loc8\#a | tatati---- | -----СатСА | gGcagcagcg | ggacgeccag | Cggcttatga |
| hungerfordiana.loc8\#b | tatait- | -catca | GGCAgCagch | ggecgeccag | cgecttatga |
| hungerfordiana.loc9\#a | tatast---- | -CATCA | gGCagcagcg | ggacgeccag | cggcttatga |
| hungerfordiana.locl6\#a | TATAAT---- | -Catca | gGCagcagch | gGgCscacag | Cagcttatga |
| hungerfordiana.locl6\#b | TATAAT---- | --CATCA | GGCAgCagch | gGgCgcceag | Cggcttatga |
| hungerfordiana.loc22\#a | tatat | -catca | GGCAGCAGCG | g gacgeccag | cgecttatga |
| hungerfordiana.loc22\#b | TATAAT---- | --CATCA | GGCATCAGCG | gGgCgcciag | CGgcttatga |
| hungerfordiana.loc23\#a | tatat | --CATCA | gGCagcagch | g gecacceag | CGgcttgta |
| hungerfordiana.loc23\#b | tatast | --CATCA | ggcagcagcg | ggacgeccag | CGGCtTGTGA |
| hungerfordiana.loc24\#a | TACAAT- | --CATCA | GGCAGCAGCG | gggcgegcag | CGGCTTGTGA |
| hungerfordiana.loc24\#b | tacast | --CATCA | GgCagcagch | gGgCGCGCAG | CGGCtTGTGA |
| hungerfordiana.loc25\#a | tatant | -Catca | GGCAGCAGCG | gGgcgeccag | CGGCttetca |
| hungerfordiana.loc25\#b | TATAAT | -catca | gGcagcagch | GGGCGCGCAG | CGGCTTGTGA |
| hungerfordiana.loc26\#a | tatat | -catca | GgCagc |  | -GGctictica |
| frequens | tattattaca | AAATACGTCA | GGC |  | ATGA |
|  | ${ }^{210}$ | ${ }_{1}^{220}$ | 230 |  | 250 |
| hungerfordiana.loc5\#a | tGAAATTA-- | -TGCTGAT | TGAACGTCTG |  |  |
| hungerfordiana.loc5\#b | TGAAATTA-- | ---TGCTGAT | TGAACGTCTG | тС |  |
| hungerfordiana.loc8\#a | TGAAATTA-- | ---TGCTGAT | TGAACGTCTG | тС |  |
| hungerfordiana.loc8\#b | TAAAATTA-- | ---TGCTGGT | TGAACGTCTG | тС |  |
| hungerfordiana.loc9\#a | TGAAATTA-- | -tGCTGAT | TGAACGTCTG |  |  |
| hungerfordiana.locl6\#a | TGAAAATA-- | -TGCTGAT | TGAACGTCTG |  |  |
| hungerfordiana.loc16\#b | TGAAAATA-- | -TGCTGAT | TGAACGTCTG |  |  |
| hungerfordiana.10c22\#a | TGAAAATA-- | -TGCTGAT | TGAACGTCTG |  |  |
| hungerfordiana.10c22\#b | TGAAAATA-- | -tgcticat | TGAACGTCTG |  |  |
| hungerfordiana.loc23\#a | TGAAAATA-- | -tGctgat | TGAACGCCTG |  |  |
| hungerfordiana. 10 23\#b | TGAAAATA-- | -TGCTGAT | TGAACGCCTG |  |  |
| hungerfordiana.loc24\#a | tGAAAATA-- | ---tGCTGAT | TGAACGCCTG |  |  |
| hungerfordiana.loc24\#b | TGAAAATA-- | -tacteat | TGAACGCCTG |  |  |
| hungerfordiana.10c25\#a | TGAAAATA-- | ---TGCTGAT | TGAACGCCTG |  | ------- |
| hungerfordiana.10c25\#b | TGAAAATA-- | ---TGCTGAT | TGAACGCCTG |  |  |
| hungerfordiana.loc26\#a | tganata-- | ---TGCTGAT | TGAACGCCTG |  |  |
| frequens | TG--TATAGA | tantgctgat | GGAACGTGTC | тСGтСтСGтС | тСGTСтСGTC |
|  |  | 270 | 280 |  |  |
| hungerfordiana.loc5\#a | ---TCCCGTT | GCCGAtcGg | GACCGCAAGA | AGCGccaccc | CGGTCGGTTG |
| hungerfordiana.loc5\#b | ---TCCCGTT | GCCAATCGGG | GACCGCAAGA | AGCGcccccc | CGGTCGGTTG |
| hungerfordiana.loc8\#a | ---тCCCGTT | GCCGATCGGG | GACCGCAAGA | AGCGCCGCCC | CGGTCGGTCG |
| hungerfordiana.loc8\#b | -TCCCGTT | GCCGATCGGG | GACCGCAAGA | agcceccecc | CGGTCGGTCG |
| hungerfordiana.loc9\#a | ---TCCCGTT | GCCGATCGGG | GACCGCAAGA | AGCGCCGCCC | CGGTCGGTTG |
| hungerfordiana.locl6\#a | ---TCCCGTT | GCCGATCGGG | GACCGCAAGA | agcceccecc | CGGTCGGTTG |
| hungerfordiana.loc16\#b | ---TCCCGTT | GCCGATCGGG | GACCGCAAGA | agcgeccecc | CGGTCGGTTG |
| hungerfordiana.loc22\#a | ---TCCCGTT | GCCGATCGGG | GACCGCGAGA | agcgeccecc | CGGTCGGTTG |
| hungerfordiana. 1oc22\#b | ---тCCCGTT | GCCGATCGGG | GACCGCAAGA | agcgeccecc | CGGTCGGTTG |
| hungerfordiana.loc23\#a | ---TCCCGTT | GCCGATCGGG | GACCGCAAGA | agcgccacce | CGGTCGGTTG |
| hungerfordiana.1oc23\#b | ---tCCCGTT | GCCGATCGGG | GACCGCAAGA | agcgeccecc | CGGTCGGTtG |
| hungerfordiana.1oc24\#a | ---TCCCGTT | GCCGATCGGG | GACCGCAAGA | agcccccecc | CGGTCGGTTG |
| hungerfordiana.1oc24\#b | ---TCCCGTT | Gccgatcgeg | GACCGcAAGA | agcceccecc | CGGTCGGTTG |
| hungerfordiana.loc25\#a | ---TCCCGTT | GCCGATCGGG | GACCGCAAGA | AGCGCCGCCC | CGGTCGGTTG |
| hungerfordiana.loc25\#b | ---тсесятt | GCCGATCGGG | GACCGCAAGG | agcaccacec | CGGTCGGTTG |
| hungerfordiana.loc26\#a | ---тСССятt | gccgatcgeg | GACCGCAAGA | agcgecgecc | cgetcgettg |
| frequens | тсGтСтсатt | gccgatcgeg | GACCGCAAGA | agcceccecc | CGGTCGGTtG |

TABLE 1. Continued

|  | ${ }_{310}^{10}$ | $\begin{gathered} 320 \\ 1 \end{gathered}$ | 330 | 340 | 350 |
| :---: | :---: | :---: | :---: | :---: | :---: |
| hungerfordiana.loc5\#a | ACCGCTCCCC | TGTTTCGGGG | TACCTAGTCT | TATCTCGCAC | TCAATACGGC |
| hungerfordiana.loc5\#b | ACCGCTCCCC | TGTTTCGGGG | TACCTAGTCT | TATCTCGCAC | TCAATACGGC |
| hungerfordiana.loc8\#a | ACCGCTCCCC | TGTTTCGGGG | TACCTAGTCT | TATCTCGCAC | TCAATACGGC |
| hungerfordiana.loc8\#b | ACCGCTCCCC | TGTTTCGGGG | TACCTAGTCT | TATCTCGCAC | TCAATACGGC |
| hungerfordiana.loc9\#a | ACCGCTCCCC | TGTTTCGGGG | TACCTAGTCT | TATCTCGCAC | TCAATACGGC |
| hungerfordiana.loc16\#a | ACCGCTCCCC | TGTTTCGGGG | TACCTAGTCT | TATCTCGCAC | TCAATACGGC |
| hungerfordiana.loc16\#b | ACCGCTCCCC | TGTTTCGGGG | TACCTAGTCT | TATCTCGCAC | TCAATACGGC |
| hungerfordiana.loc22\#a | ACCGCTCCCC | TGTTTCGGGG | TACCTAGTCT | TATCTCGCAC | TCAATACGGC |
| hungerfordiana.loc22\#b | ACCGCTCCCC | TGTTTCGGGG | TACCTAGTCT | TATCTCGCAC | TCAATACGGC |
| hungerfordiana.loc23\#a | ACCGCTCCCC | TGTTTCGGGG | TACCTAGTCT | TATCTCGCAC | TCAATACGGC |
| hungerfordiana.loc23\#b | ACCGCTCCCC | TGTTTCGGGG | TACCTAGTCT | TATCTCGCAC | TCAATACGGC |
| hungerfordiana.loc24\#a | ACCGCTCCCC | TGTTTCGGGG | TACCTAGTCT | TATCTCGCAC | TCAATACGGC |
| hungerfordiana.loc24\#b | ACCGCTCCCC | TGTTTCGGGG | TACCTAGTCT | TATCTCGCAC | TCAATACGGC |
| hungerfordiana.loc25\#a | ACCGCTCCCC | TGTTTCGGGG | TACCTAGTCT | TATCTCGCAC | TCAATACGGC |
| hungerfordiana.loc25\#b | ACCGCTCCCC | TGTTTCGGGG | TACCTAGTCT | TATCTCGCAC | TCAATACGGC |
| hungerfordiana.loc26\#a | ACCGCTCCCC | TGTTTCGGGG | TACCTAGTCT | TATCTCGCAC | TCAATACGGC |
| frequens | ACCGCTCCCC | TGTTTCGGGG | TACCTAGTCT | CGTCTCGCAC | TCAATACGGC |
|  | $\stackrel{360}{1}$ | $\begin{gathered} 370 \\ 1 \end{gathered}$ | $\begin{gathered} 380 \\ 1 \end{gathered}$ | 390 | $\begin{array}{\|c} 400 \\ 1 \end{array}$ |
| hungerfordiana.loc5\#a | CCACGGTGAC | GGCAAGAGCT | TTCAGCTCGC | CGGGTCGTCA | GGTCTAAGGA |
| hungerfordiana.loc5\#b | CCACGGTGAC | GGCAAGAGCT | TTCAGCTCGC | CGGGTCGTCA | GGTCTAAGGA |
| hungerfordiana.loc8\#a | CCACGGTGAC | GGCAAGAGCT | TTCAGCTCGC | CGGGTCGTCA | GGTCTAAGGA |
| hungerfordiana.loc8\#b | CCACGGTGAC | GGCAAGAGCT | TTCAGCTCGC | CGGGTCGTCA | GGTCTAAGGA |
| hungerfordiana.loc9\#a | CCACGGTGAC | GGCAAGAGCT | TTCAGCTCGC | CGGGTCGTCA | GGTCTAAGGA |
| hungerfordiana.loc16\#a | CCACGGTGAC | GGCAAGAGCT | TTCAGCTCGC | CGGGTCGTCA | GGTCTAAGGA |
| hungerfordiana.loc16\#b | CCACGGTGAC | GGCAAGAGCT | TTCAGCTCGC | CGGGTCGTCA | GGTCTAAGGA |
| hungerfordiana.loc22\#a | CCACGGTGAC | GGCAAGAGCT | TTCAGCTCGC | CGGGTCGTCA | GGTCTAAGGA |
| hungerfordiana.loc22\#b | CCACGGTGAC | GGCAAGAGCT | TTCAGCTCGC | CGGGTCGTCA | GGTCTAAGGA |
| hungerfordiana.loc23\#a | CCACGGTGAC | GGCAAGAGCT | CTCAGCTCGC | CGGGTCGTCA | GGTCTAAGGA |
| hungerfordiana.loc23\#b | CCACGGTGAC | GGCAAGAGCT | TTCAGCTCGC | CGGGTCGTCA | GGTCTAAGGA |
| hungerfordiana.loc24\#a | CCACGGTGAC | GGCAAGAGCT | TTCAGCTCGC | CGGGTCGTCA | GGTCTAAGGA |
| hungerfordiana.loc24\#b | CCACGGTGAC | GGCAAGAGCT | TTCAGCTCGC | CGGGTCGTCA | GGTCTAAGGA |
| hungerfordiana.loc25\#a | CCACGGTGAC | GGCAAGAGCT | TTCAGCTCGC | CGGGTCGTCA | GGTCTAAGGA |
| hungerfordiana.loc25\#b | CCACGGTGAC | GGCAAGAGCT | TTCAGCTCGC | CGGGTCGTCA | GGTCTAAGGA |
| hungerfordiana.loc26\#a | CCACGGTGAC | GGCAAGAGCT | TTCAGCTCGC | CGGGTCGTCA | GGTCTAAGGA |
| frequens | CCACGGTGAC | GGCATGAGCT | CTCAGCTCGC | CGGGTCGTCA | GGTCTAAGGA |
|  | ${ }_{410}^{10}$ | 420 <br> 1 | 430 | $\begin{array}{r} 440 \\ \hline \end{array}$ | 450 |
| hungerfordiana.loc5\#a | GCGCTGCTCT | GATTGCTCTG | TGAGCGGCGC | CGCCCCGGTG | GTTG-TGAGG |
| hungerfordiana.loc5\#b | GCGCTGCTCT | GATTGCTCTG | TGAGCGGCGC | CGCCCCGGTG | GTTG-TGAGG |
| hungerfordiana.loc8\#a | GCGCTGCTCT | GATTGCTCTG | TGAGCGGCGC | CGCCCCGGTG | GTTG-TGTGG |
| hungerfordiana.loc8\#b | GCGCTGCTCT | GATTGCTCTG | TGAGCGGCGC | CGCCCCGGTG | GTTG-TGTGG |
| hungerfordiana.loc9\#a | GCGCTGCTCT | GATTGCTCTG | TGAGCGGCGC | CGCCCCGGTG | GTTG-TGTGG |
| hungerfordiana.loc16\#a | GCGCTGCTCC | GACTGCTCTG | TGAGCGGCGC | CGCCCCGGTG | ATTG-CGTGG |
| hungerfordiana.loc16\#b | GCGCTGCTCC | GATTGCTCTG | TGAGCGGCGC | CGCCCCGGTG | ATTG-CGTGG |
| hungerfordiana.loc22\#a | GCGCTGCTCC | GATTGCTCTG | TGAGCGGCGC | CGCCCCGGTG | ATTG-CGTGG |
| hungerfordiana.loc22\#b | GCGCTGCTCC | GATTGCTCTG | TGAGCGGCGC | CGCCCCGGTG | ATTG-CGTGG |
| hungerfordiana.loc23\#a | GCGCTGCTCT | GATTGCCCTG | TGAGCGGCGC | CGCCCCGGTG | ATTG-TGTGG |
| hungerfordiana.loc23\#b | GCGCTGCTCT | GATTGCTCTG | TGAGCGGCGC | CGCCCCGGTG | ATTG-TGTGG |
| hungerfordiana.loc24\#a | GCGCTGCTCT | GATTGCTCTG | TGAGCGGCGC | CGCCCCGGTG | ATTG-TGTGG |
| hungerfordiana.loc24\#b | GCGCTGCTCT | GATTGCTCTG | TGAGCGGCGC | CGCCCCGGTG | ATTG-TGTGG |
| hungerfordiana.loc25\#a | GCGCTGCTCT | GATTGCTCTG | TGAGCGGCGC | CGCCCCGGTG | ATTG-TGTGG |
| hungerfordiana.loc25\#b | GCGCTGCTCT | GATTGCTCTG | TGAGCGGCGC | CGCCCCGGTG | ATTG-TGTGG |
| hungerfordiana.loc26\#a | GCGCTGCTCT | GATTGCTCTG | TGAGCGGCGC | CGCCCCGGTG | ATTG-TGTGG |
| frequens | GCGCCGCTCT | GACTGCTCTA | TGAGCGGCGC | CGCCCCGGTA | GTTGGTGTGG |

TABLE 1. Continued.

rememen
hungerfordiana.loc5\#a hungerfordiana.loc5\#b hungerfordiana.loc8\#a hungerfordiana.loc8\#b hungerfordiana.loc9\#a hungerfordiana.locl6\#a hungerfordiana.loc16\#b hungerfordiana.loc22\#a hungerfordiana.loc22\#b hungerfordiana.loc23\#a hungerfordiana.loc23\#b hungerfordiana.loc24\#a hungerfordiana.loc24\#b hungerfordiana.loc25\#a hungerfordiana.loc25\#b hungerfordiana.loc26\#a frequens
hungerfordiana.loc5\#a hungerfordiana.loc5\#b hungerfordiana. loc8\#a hungerfordiana.loc8\#b hungerfordiana. loc9\#a hungerfordiana.locl6\#a hungerfordiana.loc16\#b hungerfordiana.loc22\#a hungerfordiana.loc22\#b hungerfordiana.loc23\#a hungerfordiana.loc23\#b hungerfordiana. loc24\#a hungerfordiana.loc24\#b hungerfordiana.loc25\#a hungerfordiana. loc25\#b hungerfordiana.loc26\#a frequens

| 0 | 470 |  |  | - |
| :---: | :---: | :---: | :---: | :---: |
| -ATAATGGAG |  |  |  |  |
| -ATAATGGAG |  | ACCTG | GGCGCTCGAC | CCGCT-CTGC |
| -ATAATGGAG |  | GTACCTG | GC | CCGCT-CTGC |
| -ATAATGGAG |  | GTACCTG | TGCGCTCGAC | CCGCT-CTGC |
| TG |  | GTACCTG | TGCGCTCGAC | CC |
| GATAATGGAG |  | GTACCTG | GCGCTCGAC | C |
| GATAATGGAG |  | GTACCTG | TGCGCTCGAC | CCGCT-CTGC |
| GATAATGGAG |  | GTACCTG | TGCGCTCGAC | CCGCT-CTGC |
| GATAATGGAG |  | GTACCTG | TGCGCTCGAC | ССGСт-СTGC |
| -ATAACGGAG |  | GTACCTG | GCGCTCGAC | C |
| -ATAACGGAG |  | GTACCTG | TGCGCTCGAC | CG |
| - ATAACGGAG |  | GTACCTG | GCGCTCGAC | CGCTGCTGC |
| -ATGACGGAG |  | GTACCTG | TGCGCTCGAC | CCGCTGCTGC |
| - ATAACGGAG |  | -GTACCTG | TGCGCTCGAC | CCGCTGCTGC |
| -ATAACGGAG |  | GTACCTG | TGCGCTCGAC | CCGCTGCTGC |
| -ATAACGGAG |  | GTACCTG | TGCGCTCGAC | CCGCTGCTGC |
| TCAAGGAG | GCAAGGCCGG | AGGGTACCT | GCGCTCG |  |

TCGGCGGATC CGGGTGCGAT AGCTCCTGCG GTGCAAACGC AGGCCGCGA? TCGGCGGATC CGGGTGCGAT AGCTCCTGCG GTGCAAACGC AGGCCGCGA? TCGGCGGATC CGGGTGCGAT AGCTCCTGCG GTGCAAACGC AGGCCGCGA? TCGGCGGATC CGGGTGCGAT AGCTCCTGCG GTGCAAACGC AGGCCGCGA? TCGGCGGATC CGGGTGCGAT AGCTCCTGCG GTGCAAACGC AGGCCGCGA? TCGGCGGGTC CGGGTGCGAT AGCTCCTGCG GTGCAAACGC AGGCCGCGA? TCGGCGGGTC CGGGTGCGAT AGCTCCTGCG GTGCAAACGC AGGCCGCGA? TCGGCGGGTC CGGGTGCGAT AGCTCCTGCG GTGCAAACGC AGGCCGCGA? TCGGCGGGTC CGGGTGCGAT AGCTCCTGCG GTGCAAACGC AGGCCGCGA? TCGGCGGGTC CGGGCGCGAG AGCTCCTGCG GTGCAAACGC AGGCCGCGA? TCGGCGGGTC CGGGTGCGAG AGCTCCTGCG GTGCAAACGC AGGCCGCGA? TCGGCGGGTT CGGGTGCGAG AGCTCCTGCG GTGCAAACGC AGGCCGCGA? TCGGCGGGTT CGGGTGCGAG AGCTCCTGCG GTGCAAACGC AGGCCGCGA? TCGGCGGGTT CGGGTGCGAG AGCTCCTGCG GTGCAAACGC AGGCCGCGA? TCGGCGGGTT CGGGTGCGAG AGCTCCTGCG GTGCAAACGC AGGCCGCGA? TCGGCGGGTT CGGGTGCGAG AGCTCCTGCG GTGCAAACGC AGGCCGCGA? TCGGCGGCTC TGGGTGCGAC AGCTCCTGCG GTGCAAACGC AGGCCGCGA?
 GCTTAAAGA? GTCGGCC-GT A-----TGCT CGAGCA?ACC CGCCCGCTCC GCTTAAAGA? GTCGGCC-GT A-----TGCT CGAGCA?ACC CGCCCGCTCC GCTTAAAGA? GTCGGCC-AT A-TATATGCT CG?GCA?ACC CGCCCGCTCC GCTTAAAGA? GTCGGCC-AT A-TATATGCT CG?GCA?ACC CGCCCGCTCC GCTTAAAGA? GTCGGCCCAT A-----TGCT CGAGCG?ACC CGCCCGCTCC GCTTAAAGA? GTCGGCC-AT G-----TGCT CGAGCA?ACC CGCCCGCTCC GCTTGAAGA? GTCGGCC-AT G-----TGCT CGAGCA?ACC CGCCCGCTCC GCTTAAAGA? GTCGGCC-AT G-----TGCT CGAGCA?ACC CGCCCGCTCC GCTTAAAGA? GTCGGCC-AT G-_---TGCT CGAGCA?ACC CGCCCGCTCC GCTTAAAGA? GTCGGCC-AC G-----TGCT CGAGCA?ACC CGCCCGCTCC GCTTAAAGA? GTCGGCC-AT G-----TGCT CGAGCA?A-C CGCCCGCTCC GCTTAAAGA? GTCGGCC-AT G-----TGCT CGAGCA?ACC CGCCCGCTCC GCTTAAAGA? GTCGGCC-AT G-----TGCT CGAGCA?ACC CGCCCGCTCC GCTTAAAGA? GTCGGCC-AA G-----TGCT CGAGCA?ACC CGCCCGCTCC GCTTAAAGA? GTCGGCC-AA G-----TGCT CGAGCA?ACC CGCCCGCTCC GCTTAAAGA? GTCGGCC-AT G-----TGCT CGAGCA?ACC CGCCCGCTCC GCTTAAAGA? GTCGGCC-AT GCTCGCGGCT -GACCC-GCC CGCCC--T--

TABLE 1. Continued.

|  |
| :--- |
| hungerfordiana.loc5\#a |
| hungerfordiana. loc5\#b |
| hungerfordiana.loc8\#a |
| hungerfordiana.loc8\#b |
| hungerfordiana.loc9\#a |
| hungerfordiana.loc16\#a |
| hungerfordiana.loc16\#b |
| hungerfordiana.loc22 \#a |
| hungerfordiana.loc22 \#b |
| hungerfordiana.loc23\#a |
| hungerfordiana.loc23\#b |
| hungerfordiana.loc24\#a |
| hungerfordiana.loc24\#b |
| hungerfordiana.loc25\#a |
| hungerfordiana.loc25\#b |
| hungerfordiana.loc26\#a |
| frequens |

hungerfordiana.loc5\#a hungerfordiana.loc5\#b hungerfordiana.loc8\#a hungerfordiana.loc8\#b hungerfordiana.loc9\#a hungerfordiana.loc16\#a hungerfordiana.loc16\#b hungerfordiana.loc22\#a hungerfordiana.loc22\#b hungerfordiana.loc23\#a hungerfordiana.loc23\#b hungerfordiana.loc24\#a hungerfordiana.loc24\#b hungerfordiana.loc25\#a hungerfordiana.loc25\#b hungerfordiana.loc26\#a

## frequens

hungerfordiana.loc5\#a hungerfordiana.loc5\#b hungerfordiana.loc8\#a hungerfordiana.loc8\#b hungerfordiana.loc9\#a hungerfordiana.loc16\#a hungerfordiana.loc16\#b hungerfordiana.loc22\#a hungerfordiana.loc22\#b hungerfordiana.loc23\#a hungerfordiana.loc23\#b hungerfordiana.loc24\#a hungerfordiana. loc24\#b hungerfordiana.loc25\#a hungerfordiana.loc25\#b hungerfordiana.loc26\#a frequens

|  |  | 630 | 640 | -650 |
| :---: | :---: | :---: | :---: | :---: |
| GTCTTCCT-- TATTT-AATT TGTTACGCTT GGTGGCTCGT CTGTCTTATT |  |  |  |  |
| GTCTTCCT-- | TATTT-AATT | TGTTACGCTT | GGTGGCTCGT | CTGTCTTATT |
| GTCTTCCT-- | TATTT-AATT | TGTTACGCTT | GGTGGCTCGT | CTGTCTTATT |
| GTСтTCCT-- | TATTT-AATT | TGTTACGCTT | GGTGGCTCGT | CTGTCTTATT |
| GTCTTCCT- | TATTT-AATT | TGTTACGCTT | GGTGGCTCGT | CTGTCTTATT |
| GTCTTCCT- | TATTT-AATT | TGTTACGCTT | GGTGGCTTGT | CTGTCTTATT |
| GTСтTССт-- | TATTT-AATT | TGTTACGCTT | GGTGGCTCGT | CTGTCTTATT |
| GTCTTCCT | ATTT-AATT | GTTACGCTT | GTGGCTCG' | CTGTCTTATT |
| GTCTTCCT-- | TATTT-AATT | TGTTACGCTT | GGTGGCTCGT | CTG |
| GССТTCCT-- | TATTT-AATT | TGTTACGCTT | GCTGGCTCGT | CTGTCCTATT |
| GССтTCCT-- | CATTT-AATT | TGTTACGCTT | GCTGGCTCGT | CTGTCCTATT |
| GCCTTCCT-- | TATTT-AATT | TGTTACGCTT | GCTGGCTCGT | ctGtcctatt |
| GCCTTCCT-- | TATTT-AATT | TGTTACGCTT | GCTGGCTCGT | CTGTCCTATT |
| GCCTTCCT-- | TATTT-AATT | TGTTACGCTT | GCTGGCTCGT | CTGTCCTATt |
| GССтTCCT-- | TATTT-AATT | TGTTACGCTT | GCTGGCTCGT | CTGTCCTATT |
| GCCTTCCT-- | TATTT-AATT | TGTTACGCTT | GCTGGCTCGT | CTGTCCTATT |
| GTC-TCCTC | CATTTTAT | TGTTACGCT- |  | -TGTCCGAT- |

660670690 TGTCAGTTAC CGAAAAA--- ---------C AAGATTGCTT GTCGTACAAC TGTCGGTTAT CGAAAAA--- ----------C AAGATTGCTT GTCGTACAAC TGTCCGTTAT CGAAAAA--- ---------C AAGATTGCTT GTCGTACAAC TGTCGGTTAT CGAAAAG--- ----------C AAGATTGCTT GTCGTACAAC TGTCGGTTAT CGAAAAA--- ---------C AAGATTGCTT GTCGTACGAC TGTCGGTTAT CGAAAAA--- ----------C AAGATTGCTT GTCGTACAAC TGTCGGTTAT CGAAAAA--- ----------C AAGATTGCTT GTCGTACAAC TGTCGGTTAT CGAAAAA--- ---------C AAGATTGCTT GTCGTACAAC TGTCGGTTAT CGAAAAA--- ---------C AAGATTGCTT GTCGTACAAC TGTCGGTTAT CGAAAAA--A AAAACAAA-C AAGATTGCTT GTCGTACAAC TGTCGGTTAT C?AAAAAAAA AAAAC?AAAC AA?ATTGCTT GTCGT?CAAC TGTCGGTTAT CGAAAAAAAA AAAA-----C AAGATTGCTT GTCGTACAAC TGTCGGTTAT CGAAAAAAAA AAA------C AAGATTGCTT GTCGTACAAC TGTCGGTTAT CGAAAAAAAA A--------C AAGATTGCTT GTCGTACAAC TGTCGGTTAT CGAAAAAAAA A--------C AAGATTGCTT GTCGTACAAC TGTCGGTTAT CGAAAAA--- -AAACAAAAC AAGATTGCTT GTCGTACAAC ----GGTTAT CGAAAAA-CC AAAACAAAA- -----TGCTT GTCGTACAAC
 TTTGAGCGGT GGATCACTCG GCTCGTGCGT CGATGAAGAG CGCAACCAGC TTTGAGCGGT GGATCACTCG GCTCGTGCGT CGATGAAGAG CGCAGCCAGC TTTGAGCGGT GGATCACTCG GCTCGTGCGT CGATGAAGAG CGCAGCCAGC TTTGAGCGGT GGATCACTCG GCTCGTGCGT CGATGAAGAG CGCAGCCAGC TTTGAGCGGT GGATCACTCG GCTCGTGCGT CGATGAAGAG CGCAGCCAGC TTTGAGCGGT GGATCACTCG GCTCGTGCGT CGATGAAGAG CGCAGCCAGC TTTGAGCGGT GGATCACTCG GCTCGTGCGT CGATGAAGAG CGCAGCCAGC TTTGAGCGGT GGATCACTCG GCTCGTGCGT CGATGAAGAG CGCAGCCAGC TTTGAGCGGT GGATCACTCG GCTCGTGCGT CGATGAAGAG CGCAGCCAGC TTTGAGCGGT GGATCACTCG GCTCGTGCGT CGATGAAGAG CGCGGCCGGC TTT??????? ?????????? ?????????? ?????????? ?????????? TTTGAGCGGT GGATCACTCG GCTCGTGCGT CGATGAAGAG CGCAGCCAGC TTTGAGCGGT GGATCACTCG GCTCGTGCGT CGATGAAGAG CGCAGCCAGC TTTGAGCGGT GGATCACTCG GCTCGTGCGT CGATGAAGAG CGCAGCCAGC TTTGAGCGGT GGATCACTCG GCTCGTGCGT CGATGAAGAG CGCAGCCAGC TTTGAGCGGT GGATCACTCG GCTCGTGCGT CGATGAAGAG CGCAGCCAGC TTTGAGCGGT GGATCACTCG GCTCGTGCGT CGATGAAGAG CGCAGCCAGC

TABLE 1. Continued.

|  | 760 | 770 | $\xrightarrow{780}$ | ${ }_{790}^{19}$ | 8 |
| :---: | :---: | :---: | :---: | :---: | :---: |
| hungerfordiana.loc5\#a | TGCGTGAATT | AATGTGAATT | GCAGAACACA |  |  |
| hungerfordiana.loc5\#b | TGCGTGAATT | AATGTGAATT | GCAGAACACA |  |  |
| hungerfordiana.loc8\#a | TGCGTGAATT | AATGTGAATT | GCAGAACACA |  |  |
| hungerfordiana.loc8\#b | TGCGTGAATT | AATGTGAATT | GCAGAACACA |  |  |
| hungerfordiana.loc9\#a | TGCGTGAATT | AATGTGAATT | GCAGAACACA |  |  |
| hungerfordiana.loc16\#a | TACGTGAATT | AATGTGAATT | GCAGAACACA |  |  |
| hungerfordiana.loc16\#b | TGCGTGAATT | AATGTGAATT | GCAGAACACA |  |  |
| hungerfordiana.loc22\#a | TGCGTGAATT | AATGTGAATT | GCAGAACACA |  |  |
| hungerfordiana.loc22\#b | TGCGTGAATT | AATGTGAATT | GCAGAACACA |  |  |
| hungerfordiana.loc23\#a | TGCGTGAATT | AATGTGAATT | GCAGAACACA |  |  |
| hungerfordiana.loc23\#b | ?????????? | ?????????? | ?????????? |  |  |
| hungerfordiana.loc24\#a | TGCGTGAATT | AATGTGAATT | GCAGAACACA |  |  |
| hungerfordiana.loc24\#b | TGCGTGAATT | AATGTGAATT | GCAGAACACA |  |  |
| hungerfordiana.loc25\#a | TGCGTGAATT | AATGTGAATT | GCAGAACACA |  |  |
| hungerfordiana.loc25\#b | TGCGTGAATT | AATGTGAATT | GCAGAACACA |  |  |
| hungerfordiana.loc26\#a | TGCGTGAATT | AATGTGAATT | GCAGAACACA |  |  |
| frequens | TGCGTGAATT | AATGTGAATT | GCAGAACACA |  |  |

TABLE 2. Pairwise Kimura's 2-parameter distances among the G. hungerfordiana sequences.

| hunger fordiana.loc5\#a | 0.0000 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: | :---: |
| hungerfordiana.loc5\#b | 0.0085 | 0.0000 |  |  |  |  |  |  |  |  |  |  |  |  |  |  |
| hunger fordiana. $1008 \%$ a | 0.0129 | 0.0107 | 0.0000 |  |  |  |  |  |  |  |  |  |  |  |  |  |
| hunger fordiana. loc8\#b | 0.0193 | 0.0150 | 0.0085 | 0.0000 |  |  |  |  |  |  |  |  |  |  |  |  |
| hungerfordiana.loc9\#a | 0.0128 | 0.0085 | 0.0064 | 0.0107 | 0.0000 |  |  |  |  |  |  |  |  |  |  |  |
| hungerfordiana. $10016 \#$ a | 0.0324 | 0.0280 | 0.0259 | 0.0302 | 0.0236 | 0.0000 |  |  |  |  |  |  |  |  |  |  |
| hungerfordiana. loc16\#b | 0.0280 | 0.0236 | 0.0215 | 0.0258 | 0.0193 | 0.0085 | 0.0000 |  |  |  |  |  |  |  |  |  |
| hungerfordiana. $10022 \#$ a | 0.0280 | 0.0236 | 0.0215 | 0.0258 | 0.0193 | 0.0085 | 0.0042 | 0.0000 |  |  |  |  |  |  |  |  |
| hungerfordiana. 10022 *b | 0.0281 | 0.0237 | 0.0216 | 0.0259 | 0.0193 | 0.0085 | 0.0042 | 0.0042 | 0.0000 |  |  |  |  |  |  |  |
| hungerfordiana, 10023 \#a | 0.0394 | 0.0349 | 0.0328 | 0.0372 | 0.0304 | 0.0282 | 0.0238 | 0.0238 | 0.0238 | 0.0000 |  |  |  |  |  |  |
| hungerfordiana. $10023 \#$ b | 0.0481 | 0.0436 | 0.0414 | 0.0458 | 0.0391 | 0.0368 | 0.0324 | 0.0324 | 0.0324 | 0.0126 | 0.0000 |  |  |  |  |  |
| hungerfordiana. $10024 \mathrm{\# a}$ | 0.0414 | 0.0369 | 0.0348 | 0.0392 | 0.0324 | 0.0302 | 0.0258 | 0.0258 | 0.0258 | 0.0063 | 0.0147 | 0.0000 |  |  |  |  |
| hunger fordiana. $10024 \mathrm{\# b}$ | 0.0459 | 0.0413 | 0.0392 | 0.0436 | 0.0369 | 0.0345 | 0.0302 | 0.0302 | 0.0302 | 0.0105 | 0.0190 | 0.0042 | 0.0000 |  |  |  |
| hunger fordiana. 10025 \#a | 0.0415 | 0.0370 | 0.0348 | 0.0392 | 0.0325 | 0.0302 | 0.0258 | 0.0258 | 0.0259 | 0.0064 | 0.0127 | 0.0042 | 0.0084 | 0.0000 |  |  |
| hunger fordiana. $10025 \mathrm{\# b}$ | 0.0437 | 0.0392 | 0.0370 | 0.0414 | 0.0347 | 0.0324 | 0.0280 | 0.0280 | 0.0280 | 0.0085 | 0.0148 | 0.0063 | 0.0105 | 0.0021 | 0.0000 |  |
| hunger fordiana. $10026 \# \mathrm{a}$ | 0.0405 | 0.0359 | 0.0337 | 0.0382 | 0.0313 | 0.0289 | 0.0244 | 0.0244 | 0.0244 | 0.0043 | 0.0108 | 0.0022 | 0.0065 | 0.0022 | 0.0044 | 0.0000 |


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